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Sugar metabolism during postharvest storage of 'Rongrien' rambutan fruit at different stages of maturity

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Abstract

Rambutan (*Nephelium lappaceum* Linn.) variety 'Rongrien' fruit were harvested at four maturity stages based on rind ripeness color (green, orange yellow, orange red and red with green spintern tip) and fruit aril were analyzed for glucose, fructose and sucrose contents and invertase activity. Sucrose was the predominant sugar regardless of harvest maturity and increased with increasing ripeness stage. Glucose and fructose were much higher in green and orange yellow fruit than in orange red and red fruit. During storage at 25°C for 6 days, dramatic increases in glucose and fructose contents and decreases in sucrose content were noted in orange red and red fruit. Green and orange yellow fruit showed only increases in fructose content while glucose content did not vary much from the pre-storage level. Invertase activity did not correlate with changes in glucose, fructose and sucrose contents. The results indicate that sweetness of rambutan, one of the most important quality criteria, was the result of the balance of the three major sugar components dominated by sucrose in freshly harvested fruit and by glucose and fructose during postharvest storage.

Introduction

Rambutan (*Nephelium lappaceum* Linn.) of the Sapindaceae family is the third most important fruit crop in Thailand after durian and mangosteen. It is grown in about 99,000 ha, and the yielding is about 400,000 tons of fruit (Office of Agricultural Economics-Thailand, 2010). Most rambutans are produced for domestic market while only 1-2% is exported as fresh or processed fruit, mainly to Malaysia and China. 'Rongrien' is the most popular variety characterized by orange to red rind and green spintern tip at commercial harvest (Lam and Kosiyachinda, 1987; Kosiyachinda, 1988). The aril is thick, sweet, firm and juicy, and lacks sourness even in less mature fruits, unlike other varieties, and does not produce off-flavor due to over-ripening or fermentation. The aril easily separates from the small seed. A mix of green-yellow and red fruits is usually seen in local markets.

Sweetness is the most important quality criterion for rambutan and builds customer value, satisfaction and loyalty (Oude Ophuis and van Tripp, 1995). Because of this and the ease of separation of the aril from the seed, 'Rongrien' fruit has become the most sought after variety. Slightly immature fruit and over-ripe fruit are accepted by consumers (Salakpetch, 2003). Earlier studies elucidated sweetness of rambutans and its variations among different varieties and maturities and focused on analysis of total soluble solids (Wanichakul and Kosiyachinda, 1982; Ketsa and Klaewkasetkorn, 1992; Ketsa *et al*., 1995). Soluble solids content does not indicate the sugar content and the sugar components; therefore, understanding the specific sugars responsible for sweetness and the changes occurring during the postharvest period would provide clues on how to regulate such changes in order to maintain or enhance the characteristic flavor desired by consumers.

Glucose, fructose and sucrose are the major sugar components of sweet-tasting fruits including rambutan. Sweet taste could be due to one dominant sugar or a balance of the different sugar components. In some fruits, such as persimmon, fig and pomegranate, sucrose could hardly be found (Sugiyama *et al*., 1992). The purpose of this study was to determine the glucose, fructose and sucrose contents and invertase activity in 'Rongrien' rambutan fruit harvested at different stages of maturity and their changes during subsequent storage at 25°C for 6 days, which is the usual period when fruit remain highly acceptable to consumers.

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Materials and Methods

Fruit sampling

'Rongrien' rambutan fruit were harvested from a commercial orchard in Chanthaburi province, the leading production center that accounts for 45% of the total production in Thailand. Fruit were harvested at four stages of maturity based on rind and spintern color: green, orange yellow, orange red, and red with green spintern tip (Figure 1). Fruit were sorted for uniformity in size and freedom from defects, packed in rambutan cartons and transported to the postharvest laboratory at (name of institution) within 2 hours from harvest. Upon arrival, fruit were re-sorted and only damage-free fruits of uniform color and size per maturity stage were used as samples. Sixty fruit were used for each treatment per replicate. Three replicates were used for each treatment.

Storage condition

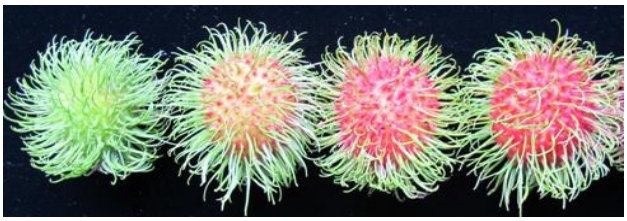
Fruit were stored in a controlled room temperature of 25±2°C and 60% RH. At 2 day intervals over 6 days of storage, fruit samples were taken for sugar analysis. Ten fruit per replicate were used at each sampling period.

Analysis of sugar components

HPLC analysis was performed to determine glucose, fructose and sucrose contents. Twenty gram (20 g) aril from the 10 sample fruit per replicate was blended with a commercial blender (Make, Model, Manufacturer, location) and the juice was squeezed out with two layers of thin cotton cloth. The juice was centrifuged at 12,000 rpm for 20 min and the supernatant was filtered through 0.45 µm filter membrane. Twenty µl of the filtrate was injected into HPLC equipped with Refractive Index detector and 25 mm x 4.6 mm APS-2 HYPERSIL column (Thermo Scientific) using deionized water:acetonitrile at 30:70 (v:v) as mobile phase at a flow rate at 0.6 ml/min (Conrad and Palmer, 1976). The analysis was done in three replicates.

Analysis of invertase activity

Invertase extraction and activity assay were done following the methods of Kubo *et al.* (2001) and Lee and Sturm (1996) with slight modifications. Mixing solution consisted of 0.6 ml of 100 mM sodium acetate buffer of pH 4.5, 0.3 ml of 100 mM sucrose and 0.1 ml rambutan juice. The solution was incubated at 37°C for 1 h, then added with dinitrosalicylic acid (DNS), and transferred to hot water bath (100°C) for 10 min. Spectrophotometer reading at 540 nm was taken. One unit of enzyme activity was expressed as one µmole of reducing sugar produced per min per mg protein. Soluble protein content was analyzed by the method of Bradford (1976). Invertase analysis was done in three replicates.



Green Orange yellow Orange red Red with green spintern tip

Figure 1. Maturity stages of 'Rongrien' rambutan based on color development of rind and spinterns (Kosiyachinda, 1988)

Data analysis

Results were analyzed by performing analysis of variance in completely randomized design and treatment mean comparison by the least significant difference test using the SAS statistical package.

Results and Discussion

Sugar components at harvest

Table 1 shows the glucose, fructose and sucrose contents of fruit aril at different stages of maturity. Sucrose was the predominant sugar regardless of harvest maturity. It progressively increased with increasing ripeness from about 1000 mg/g fresh weight (FW) in green fruits to 1980 mg/gFW in red fruits. Orange yellow and orange red fruits had intermediate sucrose contents of 1280 and 1800 g/gFW, respectively. Glucose and fructose contents showed almost opposite trend as they were higher in green and orange yellow fruits than in orange red and red fruits. Glucose was particularly high in green (960 mg/gFW) and orange yellow fruits (840 mg/gFW); it was the second most abundant sugar after sucrose. Glucose content sharply decreased by more than three-fold in orange red and red fruits, which had fructose as the second most abundant sugar, although at a lower amount (400-410 mg/gFW) than that of green and orange yellow fruits (580-600 mg/gFW).

The results show marked differences in sugar components of 'Rongrien' rambutan depending on the stage of harvest maturity. Overall, the characteristic sweet taste of freshly harvested fruit aril regardless of maturity was mainly due to sucrose content. It was highest in red fruit, which is usually the sweetest among the four maturity stages. In domestic markets, orange yellow and orange red fruits are also saleable, indicating that they have also acceptable sweet taste. This suggests that the threshold sucrose content for

Table 1. Sugar components and invertase activity at harvest of 'Rongrien' rambutan fruit of different stages of maturity

Harvest maturity	Glucose (mg/gFW)	Fructose (mg/gFW)	Sucrose (mg/gFW)	Invertase (unit/min/mg protein)
Green	960	600	1000	0.5
Orange yellow	840	580	1280	0.8
Orange red	160	400	1800	1.6
Red with green spintern tip	260	410	1980	0.5
CV (%)	11.36	2.57	3.40	8.63

Mean separation within columns by LSD, 5%.

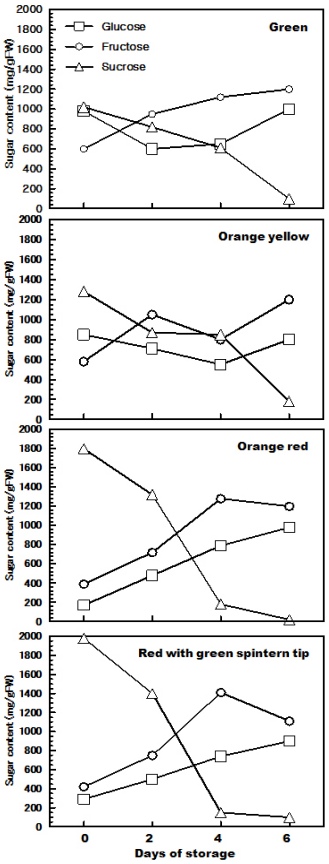


Figure 2. Changes in sugar components during storage of 'Rongrien' rambutan of different harvest maturities.

acceptable sweet taste is about 1800 mg/gFW, which was the sucrose content of orange red fruits. Both orange red and red fruits had comparable glucose and fructose contents. When glucose and fructose contents had significant contribution to sweet taste, such as in orange yellow fruits, threshold sucrose content for acceptable sweet taste would be at least 1280 mg/gFW.

Changes in sugar components during storage

Sucrose content decreased with storage (Figure 2). This was most dramatic in orange red and red fruits after 4-6 days of storage when sucrose content decreased to below 200 mg/gFW; green and orange yellow fruits exhibited such magnitude of decrease in sucrose content only after 6 days of storage. Changes in glucose content starkly differed with harvest maturity. Orange red and red fruits had increasing

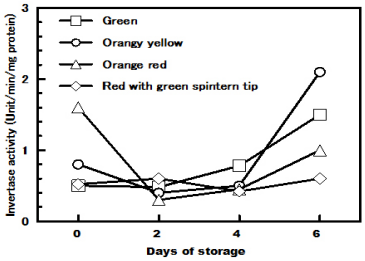


Figure 3. Invertase activity during storage of 'Rongrien' rambutan of different harvest maturities.

glucose levels with advancing period of storage so that it was about 4-5 times higher after 6 days of storage compared to that before storage. In contrast, green and orange yellow fruits had either slightly lower or comparable glucose contents relative to that before storage. Fructose content generally increased as the storage period increased regardless of harvest maturity. The increase in fructose content was more pronounced in orange red and red fruits, which had about three-fold higher fructose content after 6 days of storage than that before storage. The green and orange yellow fruits had about two-fold higher fructose content at the end of storage compared to that before storage. Fructose content at the end of storage was about 1200 mg/gFW regardless of harvest maturity.

Clearly shown from the results were the changes in sugar components responsible for sweet taste of stored fruits. Both glucose and fructose were the dominant sugars that accounted for sweetness of orange red and red fruits. In green and orange yellow fruits, it was only fructose that became the dominant sugar. In all maturity stages, sucrose became a minor sugar component, particularly at the later part of the storage period. Previously, it was found that sucrose content slightly increased with storage at 12°C (Paull and Chen, 1987). This result was not obtained in the present study which was done at a much higher storage temperature of 25°C. Yamaki (2010) further noted no net increase in glucose content, probably due to respiratory breakdown while fructose content increased presumably to serve as cellular food reserve. Both phenomena were observed in the present study but only in green and orange yellow fruits. In orange red and red fruits, the increase in both glucose and fructose contents seemed to be reflective of their formation from sucrose.

Invertase activity

At harvest, invertase activity increased with increasing stage of ripeness from green to orange red fruits; it then decreased in red fruits to levels similar to that of green fruits (Table 1 and Figure 3). During storage, invertase activity either decreased or leveled off after 2 days so that fruits from all maturity stages

had comparable invertase activities (Figure 3). This was maintained 2 days later, but at the end of the 6-day storage period, invertase activity increased in green and orange yellow fruits while that of orange red and red fruits was either lower than or comparable to the pre-storage level.

Based on their invertase activity before storage, the potential to convert sucrose to glucose and fructose was lowest in green and red fruits and highest in orange red fruits. However, this was not expressed during storage. Also, changes in invertase activity during storage were not concomitant with the changes in sucrose, glucose and fructose contents especially in orange red and red fruits. These fruits showed marked increases in glucose and fructose contents, coinciding with decreases in sucrose content, but invertase activity did not show corresponding increases. It can be speculated that the inherent invertase activity of the fruit was sufficient to catalyze the conversion of native sucrose, regardless of its concentration, to glucose and fructose and any change in invertase activity during storage was secondary rather than primary response. Previous reports also revealed that invertase activity may not be directly correlated with changes in sucrose, glucose and fructose contents of fruits during storage (Sugiyama *et al.*, 1992; Yamaki, 2010). External factors, such as storage temperature, were implicated in changes in enzyme activity that did not relate well with the levels of reaction compounds being catalyzed.

Conclusion

Sucrose was the predominant sugar responsible for sweetness of freshly harvested 'Rongrien' rambutan and was higher in fruit at more advanced stage of ripeness. In stored fruit, glucose and fructose became major sugar components, but only in orange red and red fruits, while fruits at green and orange yellow stage at harvest had fructose as the major sugar component. Changes in sugar components were not directly related with changes in invertase activity.

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